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Haplotype-based association of regulator of G-protein signaling 5 gene polymorphisms with essential hypertension and metabolic parameters in Chinese

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Abstract

Background: A recent genome-wide linkage study mapped blood pressure (BP)-related loci on human chromosome 1q and identified the regulator of G-protein signaling 5 (*RGS5*) as a candidate for regulation of BP. Thus, we assessed the relationship between *RGS5* genetic polymorphisms and essential hypertension (EH) in Chinese.
Methods: A total of 906 patients with EH and 894 age- and gender-matched normotensive (NT) controls were enrolled. Sixteen single nucleotide polymorphisms (SNPs) in *RGS5* were genotyped.
Results: There were no significant differences in the overall distributions of the genotypic and allelic frequencies for each SNPs between the two groups. However, in haplotype analysis, significant differences for the overall distributions were noted for four haplotypes constructed by five SNPs (rs12041294C/T, rs10917690A/G, rs10917695T/C, rs10917696T/C and rs2662774G/A), viz. H_2 (C–A–C–T–A) ($p=0.038$), H_5 (C–G–T–T–G) ($p=0.001$), H_6 (T–G–C–T–A) ($p=0.021$) and H_{12} (T–A–T–T–G) ($p=0.023$). Serum concentrations of high- and low-density lipoprotein cholesterol showed significant associations with haplotypes revealed by a global test ($p=0.0001$ and 0.0309).
Conclusions: Multiple SNPs in combination in *RGS5* may confer risk for hypertension. Our results also lend support for the effect of *RGS5* SNPs on lipid metabolism. Further studies are warranted to find the causal SNPs in *RGS5* for EH.
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Keywords: haplotypes; hypertension; polymorphism; RGS5.

Introduction

Essential hypertension (EH) is a complex polygenic disease determined by both genetic and environmental factors (1). Genetic factors play an important role in determining susceptibility for EH. Elucidation of genetic variants contributing to an individual's susceptibility is important, but remains elusive due primarly to the heterogeneitic and polygenic nature of EH. Genetic variants have been shown to be associated with EH or blood pressure (BP), and thus warrants further investigation among different populations.

The regulator of G-protein signaling (protein: RGS and gene: *RGS*) are proteins modulating the G-protein-coupled receptor (GPCR), induced signaling by enhancing hydrolysis of guanosine triphosphate (GTP). RGS plays a critical role in cardiovascular signal transduction. RGS proteins form a superfamily containing at least 25 members; each RGS is encoded by a different gene with different function (2–5). In the cardiovascular system, regulator of G-protein signaling 5 (RGS5) is an important signaling regulator. The physiological and pathological functions of RGS5 have been studied recently (6–8). A genome-wide linkage and candidate association study has identified a relation between human *RGS5* and increased BP in an American population (9). The expression of *RGS5* was downregulated in cerebral microcapillaries of stroke-prone spontaneously hypertensive rats (10), and in resistant arteries taken from two animal models of hypertension (11). In addition, a *RGS5* knockout mouse model displayed lower BP (12). These lines of evidence suggest the involvement of RGS5 in hypertension with an unknown mechanism. However, a literature search did not find any recent reports that assess the contribution of *RGS5* variations to susceptibility of Chinese to hypertension. Therefore, we investigated the relationship of *RGS5* variations with hypertension and intermediate phenotype in a large group of Han Chinese.

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Study population and methods

Study population

All subjects in this study were of Han Chinese descent and currently residing in the Shanghai area. A total of 1800 unrelated subjects were recruited, including 906 patients with EH and 894 normotensive (NT) controls. For each subject, clinical history and biochemical data were recorded. The study protocol was reviewed and approved by the Ethics Committee of Shanghai Ruijin Hospital, and written informed consent was obtained from each subject.

BP was measured by trained and certified hospital nurses using a mercury sphygmomanometer, according to a standard protocol recommended by the American Heart Association (13). BP was measured three times after rest in the seated position for at least 10 min and before breakfast in the morning. The mean of the three readings was used. Hypertension was defined as systolic blood pressure (SBP) $\geq$140 mm Hg or diastolic blood pressure (DBP) $\geq$90 mm Hg on three separate days, or the current use of antihypertensive drugs. In all patients, the onset of hypertension was before 70 years of age and secondary causes were ruled out through extensive clinical and biochemical studies. All NT controls were matched with EH patients for age, gender and area, with a SBP $<$130 mm Hg and DBP of $<$85 mm Hg. They did not have a history of familial hypertension, hepatic disease, renal insufficiency and diabetes.

Phenotype measurements

Body mass index (BMI) was calculated from body weight and height, with weight in kilograms divided by the square of height in meters. Height and weight was recorded without shoes and with subjects wearing light indoor clothing. Plasma triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) concentrations were determined enzymatically using available kits and an auto analyzer in Ruijin Hospital. Concentrations of low-density lipoprotein cholesterol (LDL-C) were estimated using the Friedewald equation (14).

Selection of single nucleotide polymorphisms (SNPs)

Haplotype Mapping Project data (www.hapmap.org) tagger, developed by de Bakker et al. (15), was used for selection of tag SNP. Sixteen tag SNPs in *RGS5* with minor allele frequencies of $>$5% in the Han Chinese (Beijing, China) population were selected to capture the common variation in or around this gene, with a minimum r^2 of 0.80. The information of the 16 tag SNPs in *RGS5* is shown in Table 1.

Genotyping

Genomic DNA was extracted from leukocytes in peripheral blood sample using a standard phenol-chloroform method. All genotyping was performed using TaqMan assays and followed by allelic discrimination with the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The *RGS5* SNP Taqman probes and primers were obtained from Applied Biosystems Assay-by-Design Service for SNP genotyping. The Universal PCR Master Mix from Applied Biosystems was used in a 5-μL total reaction volume with 10 ng DNA per reaction. Five percent of all genotypes were repeated in independent PCR reactions to check for consistency and to ensure intra- and inter-plate genotype quality control. On average, genotyping was successfully completed in $>$97% subjects.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was assessed in each group using the χ^2-test. Allele and genotype frequencies for each SNP comparing hypertensive and NT individuals were completed using SHEsis (16) software, available at http://analysis.bio-x.cn/myAnalysis.php. For quantitative phenotypes, an ANOVA F test was used to examine the association between a genotype and phenotype. A two sided probability of $p \leq 0.05$ was considered statistically significant, unless otherwise indicated. The degree of linkage disequilibrium (LD) between SNPs within a gene was performed with Haplo view 4.1 software available at www.broad.mit.edu/mpg/haploview. Multifactor dimensionality reduction (MDR) software package (v.2.0.0), available at http://www.epistasis.org, was used to evaluate the interaction information of all SNPs tested with $D' \leq 0.8$, and to select the five best SNPs with strong synergism. To estimate haplotype frequencies in each group and test for association with phenotypes and EH, we employed haplo.score and haplo.glm functions implemented in the Haplo.stats program (17, 18) (www.r-project.org). In addition, multiple-test was adjusted by using the false discovery rate (FDR) method with an

Table 1 Description of the selected SNPs in *RGS5*.

SNP	Contig position	Region	Allele[a]	MAF[b]
rs1056515	161379884	3′UTR	G/T	0.480
rs3806366	161381945	3′ UTR	A/G	0.253
rs2841997	161386863	Intron4	G/A	0.239
rs16849973	161399331	Intron2	A/G	0.268
rs1876451	161399427	Intron2	C/T	0.170
rs6704267	161401028	Intron2	A/C	0.487
rs12029882	161406986	Intron1	A/G	0.169
rs12041294	161408115	Intron1	C/T	0.442
rs10917690	161408792	Intron1	A/G	0.476
rs12035879	161409179	Intron1	G/A	0.444
rs10917695	161415352	Intron1	T/C	0.336
rs10917696	161415949	Intron1	T/C	0.087
rs7528044	161416433	Intron1	A/C	0.157
rs4657247	161418521	Intron1	C/T	0.210
rs10799902	161426306	Intron1	G/A	0.092
rs2662774	161427644	Intron1	G/A	0.081

UTR, untranslated region; MAF, minor allele frequency; [a]major allele/minor allele; [b]minor allele frequency in controls.

FDR q-value threshold of 0.20 as suggested by Smith et al. (19).

Results

The baseline characteristics of the study population are shown in Table 2. There were no significant differences in age, gender, and serum concentrations of TC and LDL-C between patients with EH and NT controls.

Single-point association analysis

There were no deviations from HWE for all studied polymorphisms in controls ($p > 0.05$). As shown in Supplementary Table 1, no significant differences were noted in the distributions of the genotypic and allelic frequencies for all 16 SNPs between patients with EH and NT controls.

Haplotype analysis

For adjacent SNPs in strong LD ($D' > 0.8$), we chose only one SNP for subsequent analyses with the MDR program. This was used to generate all possible combinations with genetic interaction. In this study, the Tuned Relief filter function was employed to select the top-five potential SNPs with strong synergism including rs12041294 C/T, rs10917690 A/G, rs10917695 T/C, rs10917696 T/C and rs2662774 G/A. These had a testing accuracy of 0.5572 and cross-validation consistency of 10 out of 10. Table 3 shows the results of the haplotype analysis evaluated by the Haplo.score and Haplo.glm modules. Individual analysis identified significant associations of four haplotypes, H_2 (C–A–C–T–A) ($p = 0.038$), H_5 (C–G–T–T–G) ($p = 0.001$), H_6 (T–G–C–T–A) ($p = 0.021$) and H_{12} (T–A–T–T–G) ($p = 0.023$), with EH. Association results for haplotypes seen in H_5, H_6 and H_{12} had an independent effect on decreasing risk for hypertension after adjustment for age, gender and BMI, with an adjusted odds ratios of 0.357 for H_5 (95% CI: 0.194–0.655), 0.329 for H_6 (95% CI: 0.129–0.842) and 0.346 for H_{12} (95% CI: 0.138–0.864), respectively. H_2 had an independent effect on increasing risk for EH after adjusting for confounding factors, with an adjusted odds ratio of 1.727 (95% CI: 1.029–2.897). Even after FDR adjustment for multiple testing, these associations remained significant, with FDR q-values less than the suggested threshold 0.20.

Association for the RGS5 SNPs and metabolic parameters in NT controls

The phenotype-genotype association was analyzed in NT controls only, since some clinical examinations were likely affected by drug treatment in patients with

Table 2 Demographics of the study participants.

Variables	Hypertensive patients (n = 906)	Normotensive controls (n = 894)	p-Value
Gender (men/women)	454/452	440/454	0.705
Age, years	51.7 ± 8.9	51.3 ± 6.9	0.331
BMI, kg/m^2	25.9 ± 4.2	23.1 ± 2.9	< 0.0001
SBP, mm Hg	149.9 ± 21.5	112.8 ± 10.0	< 0.0001
DBP, mm Hg	93.6 ± 13.5	75.2 ± 6.8	< 0.0001
TC, mmol/L	4.89 ± 0.93	4.83 ± 0.95	0.181
TG, mmol/L	2.05 ± 1.23	1.27 ± 0.77	< 0.0001
LDL-C, mmol/L	3.18 ± 0.86	3.18 ± 0.92	0.984
HDL-C, mmo/L	1.32 ± 0.51	1.48 ± 0.33	< 0.0001

Data are expressed as mean ± SD. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Table 3 Distribution of haplotypes in patients with EH and NT subjects.

Haplotype[a]	EH	NT	p-Value[c]	FDR q	OR [95% CI][c]
H_1 C–A–C–C–G	0.045	0.058	0.312	0.490	0.766 [0.458–1.283]
H_2 C–A–C–T–A	0.048	0.028	0.038	0.106	1.727 [1.029–2.897]
H_3 C–A–C–T–G	0.114	0.095	0.737	0.737	0.934 [0.629–1.388]
H_4 C–A–T–T–G	0.287	0.261	0.353	0.485	0.873 [0.656–1.162]
H_5 C–G–T–T–G	0.034	0.076	0.001	0.011	0.357 [0.194–0.655]
H_6 T–G–C–T–A	0.013	0.036	0.021	0.231	0.329 [0.129–0.842]
H_7 T–G–C–T–G	0.066	0.046	0.617	0.754	0.865 [0.490–1.527]
H_9 C–G–C–T–G	0.015	0.013	0.662	0.728	0.797 [0.289–2.201]
H_{10} T–G–C–C–G	0.026	0.015	0.272	0.499	1.626 [0.684–3.867]
H_{11} T–A–C–T–G	0.010	0.016	0.134	0.295	0.299 [0.061–1.453]
H_{12} T–A–T–T–G	0.008	0.050	0.023	0.084	0.346 [0.138–0.864]
H_8 T–G–T–T–G[b]	0.303	0.273			BASE

[a]Combinational SNPs were arranged in the order as following: rs12041294 C/T, rs10917690 A/G, rs10917695 T/C, rs10917696 T/C and rs2662774 G/A; [b]H_8 T–G–T–T–G was chosen as a reference haplotype in the analyses; [c]OR [95% CI]; p-values were calculated after adjustment for age, gender, BMI.

EH. We found that BMI was significantly higher for the rs12041294 minor group (23.59±2.93 kg/m^2) compared with the major (22.90±3.15 kg/m^2, p=0.019) and heterozygous (22.99±2.77 kg/m^2, p=0.027) groups. The serum concentration of TG was significantly higher for the rs2841997 major (1.29± 0.79 mmol/L, p=0.025) and heterozygous (1.28± 0.77 mmol/L, p=0.035) groups compared with the minor group (1.05±0.60 mmol/L); the concentration of TC was significant higher for the rs2841997 heterozygous (4.81±0.96 mmol/L, p=0.022) group than the minor group (4.62±0.95 mmol/L).

Association for the RGS5 haplotypes and metabolic parameters in NT controls

Table 4 lists the association of haplotypes with blood lipid concentrations. In NT controls, serum concentrations of HDL-C and LDL-C showed significant association with haplotypes (global p=0.0001 and 0.0309). Individual analysis found a significant association between H_1 and HDL-C (Hap-score=–2.87, p=0.004). Similar findings were seen in H_3 (Hap-score=2.48, p=0.013), H_5 (Hap-score=2.35, p=0.019), H_6 (Hap-score=–2.16, p=0.031) and H_{12} (Hap-score=2.58, p=0.01). For H_{12}, there was a significant association with TC (Hap-score=2.40, p=0.016) and LDL-C (Hap-score=2.97, p=0.003). In addition, significant associations between H_{11} and SBP (Hap-score=2.09, p=0.037), H_3 and DBP (Hap-score=–2.02, p=0.043) were found.

Discussion

The candidate gene approach may not replace the genome-wide scan strategy in detecting the genetics of complex traits. However, it is an important alternative strategy, especially when the population studied is large enough, and the candidate genes are selected on the basis of strong biological hints (20). It has been proposed that to generate robust data, a much larger sample involving 1000 subjects in each group might be required (21). Our sample size included 906 cases and 894 controls. To the best of our knowledge, this is the first case-control study to date focusing on *RGS5* polymorphisms and susceptibility to EH in Han Chinese.

A recent study documented that the 1q23-32 regions harbor multiple EH-susceptible genes that affect BP, and provided evidence of an independent association between three genes in this region and BP. *RGS5* was one of the three genes (9). In addition, several genome linkage studies of BP and EH in humans, as well as in rat and mouse models of hypertension, suggest that chromosome 1q23-q32 regions were likely to contain EH-susceptible loci (22–27). Along with these lines of evidence, we consider *RGS5* as a candidate gene for investigating genetic susceptibility to EH in a large Han Chinese population. Chang et al. (9) investigated multiple genes with respect to susceptibility to EH and found that a total of 13 SNPs in *RGS5* were assigned in two LD blocks. Therefore, eight SNPs showed significant associations with BP in at least one of six sample groups studied. In contrast, in the present study, all 16 SNPs covered these two LD blocks, but none showed any significant association with EH in single point analysis. In addition, some SNPs, such as rs3806366, showed great genetic heterogeneity. For example, the rs3806366 A allele frequency was 0.75 in this study. This is between that of European-Americans (0.91) and African-Americans (0.50) in the study of Chang et al. (9). In view of genetic heterogeneity among different ethnic populations, it would be important to construct a database of polymorphisms responsible for EH for each race or ethnic group.

As we previously indicated, haplotype analysis could provide more information about the effect of genetic interaction, especially when these alleles have a synergistic effect (28). Therefore, we constructed haplotype models using the six MDR derived polymorphisms as a whole. It is generally believed that haplotype analysis, which studies single genetic variant in their combination simultaneously, has a higher complexity level than single-locus analysis. Also, this has more power for assessing the association between candidate genes and complex diseases (29). This approach does not require the causal variants be identified or directly tested, but rather has the potential to highlight physical regions that harbor putative disease-associated variants (30). In this study, our haplotype-based association study revealed some predominance of SNP combinations associated with disease status, and conferred increased or decreased

Table 4 Blood lipid parameters by individual haplotypes.

Haplotype[a]	TG		TC		HDL-C		LDL-C	
	Hap-score	p-Value	Hap-score	p-Value	Hap-score	p-Value	Hap-score	p-Value
H_1 C-A-C-C-G	–1.62	0.106	1.57	0.117	–2.87	0.004	–1.35	0.179
H_3 C-A-C-T-G	–0.42	0.674	1.43	0.153	2.48	0.013	1.22	0.221
H_4 C-A-T-T-G	1.89	0.059	–1.91	0.056	–1.58	0.115	–0.63	0.528
H_5 C-G-T-T-G	–0.21	0.831	1.11	0.269	2.35	0.019	1.08	0.278
H_6 T-G-C-T-A	–1.62	0.106	–0.74	0.458	–2.16	0.031	–1.89	0.059
H_7 T-G-C-T-G	–0.60	0.547	–1.18	0.239	–0.58	0.564	–1.65	0.099
H_{12} T-A-T-T-G	0.06	0.950	2.40	0.016	2.58	0.010	2.97	0.003
H_8 T-G-T-T-G	–0.55	0.582	–0.26	0.791	–1.67	0.095	–1.33	0.184

Haplotypes with frequencies <0.05 were not included in the Table. [a]Combinational SNPs were arranged in the order as following: rs12041294 C/T, rs10917690 A/G, rs10917695 T/C, rs10917696 T/C and rs2662774 G/A.

risk for EH. For example, haplotypes H_5 (C–G–T–T–G), H_6 (T–G–C–T–A), H_{12} (T–A–T–T–G) had a 64.3%–67.1% decreased risk for EH, while H_2 (C–A–C–T–A) had a 72.7% increased risk. If the polymorphisms derived by MDR function were actually involved, or were markers in strong linkage with other functional polymorphisms involved in the pathogenesis of EH, two possible inferences can be drawn. One is that rs10917696 is unlikely to play a leading role since the T allele harbors both protective and risk-conferring haplotypes. Another inference is the genetic interaction between rs12041294 C/T and rs10917690 A/G polymorphisms, because haplotypes with one or two mutant allele(s), such as C–G, T–A, T–G in these two SNPs showed decreased risk, whereas haplotypes with two wild alleles (C–A) resulted in increased risk for EH. Because statistical interaction may not automatically imply biological interaction, haplotype analysis may represent the first step in providing clues for directing future research.

We performed phenotype-genotype associations among NT controls only. This is because these clinical phenotypes might be confounded by drug regimens in patients with EH. Of note, individuals carrying the rs12041294 2/2 genotype had significantly higher BMI than those carrying the 1/1 and 1/2 genotype. Also, TG concentrations were significantly higher for individuals carrying the rs2841997 1/1 and 1/2 genotype compared with those carrying the 2/2 genotype. A similar trend was noted for TC. Expanding these findings, significant associations were also found between several haplotypes and blood lipids. Our results suggest that RGS5 might play a role in the control of body weight and lipid metabolisms, in agreement with previous studies. Cho et al. reported that *Rgs5*-/- mice weighed less than littermate controls. The relative fat content per gram of weight of the littermate controls was significantly higher than that of the *Rgs5*-/- mice (12). In addition, RGS5 has been reported to be expressed in mouse adipocytes isolated from subcutaneous and intra-abdominal fat. Given that the association analyses of our quantitative trait were based on the control group, and only limited SNPs showed marginally significant associations with lipid parameters, further studies will be required in the population at large to test its effect on body weight and lipid metabolism.

Despite the strengths of the present study, including the large sample size and systematic selection of *RGS5* polymorphisms, our study should be interpreted within the context of its limitations. First, for the case-control nature of this study design, it inevitably suffers from the limitations of this type of study; i.e., the inability to prove the existence of a causality relationship. Second, we genotyped only 16 common polymorphisms in *RGS5,* and did not examine other genes/polymorphisms that might be associated with hypertension. Also, the polymorphisms selected do not cover the genes fully and extensively. Third, other risk factors or intermediate phenotypes, such as lifestyles (salt consumption and physical activity etc.) were unavailable for this analysis. Therefore, our results should be considered preliminary. In addition, confirmation from a biological or clinical point of view is critical.

In conclusion, the present study suggests multiple variants in combination in *RGS5,* rather than a single SNP, may alter the risk for hypertension. Further studies are warranted to find the causal variations of *RGS5* in EH, and to prove the effect of *RGS5* gene variants on lipid metabolism.

Supplementary data associated with this article can be found in the online version at: http://www.reference-global.com/doi/suppl/10.1515/CCLM.2009.215.

Conflict of interest statement

We did not accept any funding or support from an organization that may in any way gain or lose financially from the results of our study. We have not been employed by an organization that may in any way gain or lose financially from the results of your study. We do not have any other conflicting interests.

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